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[From *The Johns Hopkins Hospital Bulletin*, No. 76, July, 1897.]

ON THE BLOOD-PRESSURE-RAISING CONSTITUENT OF THE SUPRARENAL CAPSULE.*

BY JOHN J. ABEL, M. D., AND ALBERT C. CRAWFORD, M. D.

[*From the Pharmacological Laboratory of the Johns Hopkins University.*]

Both clinical experience and laboratory research have shown that the suprarenal capsule is an organ of vital importance.

Physiologists† have proved that a very small quantity of an aqueous extract of the medullary substance raises the blood pressure to a great height above the normal. It has also unequaled power in reviving a poisoned heart. Gottlieb,‡ for example, has shown that it will revive the heart of a rabbit which has practically stopped beating in consequence of an intravenous injection of chloral hydrate.

Bates§ applied an aqueous solution to the eye and found that it exerted a marked vaso-constricting action. In numerous cases of congestion a small quantity dropped into the conjunctival sac brought about an immediate pallor, lasting for some time. According to this writer the extract is very useful in prolonged operations, for, when repeatedly applied, hæmorrhage is prevented and cocaine anæsthesia is in consequence indefinitely prolonged. As the result of his two years' use of the extract Bates concludes "that within the limits of its sphere of activity there is absolutely no other substance which can take its place."

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*Read before the Association of American Physicians, May 6, 1897.

†Schäfer and Oliver, Journal of Physiology, xvi (1894) and xvii (1895); Szymonowicz and Cybulski, Wien. med. Wochenschrift, 1896, No. 6.

‡Arch. f. exp. Pathol. u. Pharmakol., xxxviii, 106.

§New York Medical Journal, lxiii (1896), 647.

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Other experiments[*] go to show that the aqueous extract is a powerful poison when injected directly into the circulation and may lead to fatal results.

The various extracts that have been used in these experiments were mixtures of unknown substances, and it is as yet an unsolved question whether the various actions at present ascribed to the gland are due to one and the same substance.

We are at present interested in the isolation of the blood-pressure-raising constituent, for in a purified state, separated from all other constituents, it might become a therapeutic agent of great importance.

On the chemical side but little advance has been made over Vulpian's[†] striking original contribution more than forty years ago. Vulpian observed that the juice expressed from the suprarenal capsule of many different animals behaved in a striking manner toward ferric chloride and toward solutions of iodine, giving with the former reagent an emerald green color, and with the latter a beautiful rose carmine tint. No other tissue of the body, so far as investigated by Vulpian, gave these reactions.

Virchow[‡] substantiated Vulpian's statements, but added nothing new. A year after Vulpian's first announcement his second paper appeared in conjunction with Cloëz,[§] verifying and extending his first observations and stating his failure to isolate the chromogenic substance or substances to which the above reactions are due.

Equally unsuccessful were Arnold[||] and Holm.[**] Krukenberg,[††] years after, repeated the work of Arnold and came to the conclusion that the chromogen of the suprarenal capsule is a non-volatile, nitrogenous and ferruginous organic acid which is probably related to the turacin of the musophagidæ and to chlorophyll. He assumed that the substance giving the green color with ferric chloride is not the chromogenic substance of Vulpian, but more likely pyrocatechin accompanying the chromogen. Attention being thus attracted to the possible occurrence of pyrocatechin in the suprarenal capsule, others took up the subject.

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[*] Foa and Pellacani, cited in Maly's Jahresb. d. Thier-Chemie, xiii, 129; Marino-Zuco, ibid., xviii, 231; Gourfein, Rev. Méd. de la Suisse Romande, Oct. 20, 1895; Compt. rend., cxxi (1895), 311-314.

[†] Note sur quelques reactions propres à la substance des capsules surrénales, Compt. rend., xliii (1856), 663-665.

[‡] Virchow's Archiv, xii (1857), 481-483.

[§] Compt. rend., xlv (1857), 340-343.

[||] Virchow's Archiv, xxxv (1866), 64-107.

[**] Journ. f. prakt. Chemie, c (1867), 150.

[††] Virchow's Archiv, ci (1885), 542-591.

Brunner* found that an alcoholic extract can be made to give nearly all of the reactions of pyrocatechin; thus, it gives the well-known green color with ferric chloride, passing into a fine red on the addition of ammonium tartrate and a few drops of an alkali; it reduces silver nitrate at room temperature, and Fehling's solution on boiling. The addition of an alkali soon causes it to take on a dark brown color, lead acetate gives a precipitate, sodium nitroprusside and very dilute ammonia give a wine-red color. All of the above reactions being given also by pyrocatechin, Brunner concluded that Krukenberg was right in his belief that pyrocatechin is present in the suprarenal gland.

After the discovery of the blood-pressure-raising property of the suprarenal gland, B. Moore,† working in Schäfer's laboratory, came to the conclusion that Vulpian's chromogen and the blood-pressure-raising constituent are identical. He based his opinion on the fact that chemical operations which destroy the color reactions by oxidizing the reducing agent appear also to destroy the blood-pressure-raising constituent. That the solubilities of the active principle are the same as those of the reducing agent appears also to support this opinion.

Fraenkel‡ worked with residues obtained with the help of alcohol and acetone as solvents. These residues raise the blood pressure and do not contain pyrocatechin, and on the strength of the ferric chloride reaction and its reducing power he concludes that the essential principle of the residue is a nitrogenous derivative of the *ortho*dihydroxy-benzene series.

According to Fraenkel both Krukenberg and Brunner are wrong in their opinion that the suprarenal gland contains pyrocatechin. Fraenkel concludes, like Moore, that the blood-pressure-raising constituent and Vulpian's chromogen are one and the same substance.

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* Schweizer. Wochenschr. f. Pharmacie, xxx (1892), 121–123.

† Journal of Physiology, xvii (1895), Proc. Physiol. Soc., p. xiv.

‡ Wiener med. Blätter, 1896, No. 14–16.

More recently still, Mühlmann* has attempted to prove that the blood-pressure-raising constituent is a pyrocatechin derivative. He asserts that on boiling fresh suprarenal capsules with dilute hydrochloric acid the active principle is decomposed and the pyrocatechin which is split off may be taken up with ether. Mühlmann has not, however, furnished conclusive chemical proofs for his assertion. Both Mühlmann and Brunner might have settled this point by precipitating the supposed pyrocatechin with lead acetate and analyzing the lead salt thus obtained. Pyrocatechin may be present in small amounts in the gland, but no proof of this has yet been furnished.

There is therefore at present great diversity of opinion as to the chemical character of the blood-pressure-raising constituent of the gland.

Whatever the probability may be of the correctness of this or that view, it is to be noted that all of the above-named investigators have based their conclusions on reactions made with aqueous, alcoholic or acetonic extracts; none of them have even roughly isolated a definite chemical compound. The subject is one of great difficulty, and our own work is at present merely preliminary, but we have arrived at the following conclusions which we believe to be, borne out by our experiments.

First, we have found by isolating the blood-pressure-raising constituent in the form of a benzoyl compound and decomposing it, that the active principle is a substance with basic characteristics and that it must in all probability be classed with the pyrrol compounds or with the pyridine bases or alkaloids.

Second, that pyrocatechin cannot be split off from the isolated active compound by boiling with acids, as has been asserted.

Third, we have found that a carmine-red pigment can be separated from the sulphate of the active principle without destroying its power to raise the blood pressure.

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* Deutsche med. Wochenschr., 1896, No. 26, 409–411.

Fourth, in addition to this, we have isolated from the crude benzoyl product a volatile basic body which fumes in the air and which emits an odor very much like that of coniine.

METHOD OF ISOLATING THE ACTIVE PRINCIPLE IN THE FORM OF A BENZOYL COMPOUND.

We have used sheep's glands in large quantity. The medullary substance of the fresh glands was scraped out, dried on the water-bath at 60° C., ground up finely, and extracted with ether for several days until the fats and the substance known as Manasse's jecorin were removed. In this way a fine dry powder of a grayish white appearance is obtained, the aqueous extract of which is very active in raising the blood pressure.

With 100 grammes or more of this powder, representing one kg. in weight or about 1000 fresh glands, we proceeded as follows: The powder is repeatedly extracted with warm water acidulated with a few drops of dilute sulphuric acid until the gland has yielded up all of its chromogenic substances, as tested by Vulpian's reactions. This aqueous extract is evaporated on the water-bath to a small bulk, a large excess of strong alcohol is then added and the whole allowed to stand 24 hours, by which time the proteids have all settled out. The alcoholic fluid is then filtered and the greater part of the alcohol removed by distillation; the remainder is driven off on the water-bath. The entire removal of the alcohol is necessary, and in order to avoid too great concentration of the acid fluid, which seems to destroy some of the active principle, water has now and then to be added. The brownish fluid when filtered gives all the well-known reactions characteristic of Vulpian's chromogen, and on neutralizing the sulphuric acid we found the filtrate to be physiologically very active.

This filtered fluid is next treated with the proper quantity of benzoyl chloride and sodium hydrate according to the Baumann–Schotten method of forming a benzoyl compound. There results a tarry substance of a light yellow color which sticks to the sides of the separator funnel and which is collected and washed until the wash fluid no longer gives an alkaline reaction. It is next dissolved as far as possible in alcohol and the alcohol evaporated. The residue is then treated with ether, which takes up the greater part of it, the ethereal solution is filtered and the ether driven off.

The residue, which is of tarry consistence and amber color, is taken up in strong alcohol from which all basic substances have been removed by distilling from tartaric acid. It is then boiled for two hours with animal charcoal previously well washed with purified alcohol. After filtration and the evaporation of the alcohol a considerable quantity of crystalline residue is obtained which is found to consist of the benzoyl compound of the active principle contaminated with other benzoyl derivatives and which we may call the crude benzoyl product. It is insoluble in water, alkalies or acids of ordinary strength, is soluble in alcohol, ether, glacial acetic acid and concentrated sulphuric acid.

It is of interest in this connection to note that benzoyl chloride effects the complete removal of the blood-pressure-raising constituent from an aqueous solution. This is proved by the fact that the filtrate from the insoluble benzoate no longer gives the characteristic chemical reactions of the active principle, and that chemical manipulations no longer yield a blood-pressure-raising constituent. Indeed, an injection of a sufficient amount of the material left after the sodium benzoate and the excess of alkali have been removed, causes a sharp and sudden fall of pressure instead of a rise.

It was not until long after we had made use of benzoyl chloride as a precipitant of the active principle that we obtained access to Fraenkel's* paper. From it we learned that a syrupy substance is thrown out when benzoyl chloride is shaken up with a pyridine solution of an extract of the gland. Fraenkel did not succeed in crystallizing this benzoyl compound, nor did he attempt to decompose it in order to ascertain whether it was in reality a benzoate of the blood-pressure-raising principle.

METHODS OF DECOMPOSING THE BENZOYL COMPOUND.

A quick method always used by us as a qualitative test is as follows: The compound is dissolved in as little concentrated sulphuric acid as possible, crushed ice is slowly added, and the benzoic acid which has been split off is shaken out with ether. On neutralizing the solution we found it to give the reactions characteristic of aqueous solutions of the medullary substance. We have not considered this method adapted to the decomposition of large quantities of the product.

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* Wien. med. Blätter, 1896, No. 14–16.

Decomposition of this benzoyl product was effected by means of an alcoholic solution of sodium alcoholate, and also with strong hydrochloric acid and alcohol, but these methods were found to be less satisfactory in practice than the following, which is the method on which we now rely. The benzoyl compound is dissolved in glacial acetic acid, the solution heated to near the boiling point, and an equal volume of a 25 per cent. solution of sulphuric acid actively boiling is slowly poured into the hot acetic acid solution, the whole being shaken meanwhile. The flask is now attached to a back-flow condenser and heated on a small flame for about ten minutes.

The solution is next diluted with water and allowed to cool, the benzoic acid, which has been thrown out, is filtered off and the rest is removed with the help of ether.

Mixed with the benzoic acid which has been removed by filtration is found a dark brittle substance which softens on the water-bath, and which we assume to be a benzoyl compound of unknown composition which was apparently unaffected by the dilute sulphuric acid. On dissolving it in concentrated sulphuric acid and diluting with crushed ice, the resulting fluid gives none of the reactions characteristic of suprarenal extracts.

After decomposing the benzoyl product with hot dilute sulphuric acid and removing the benzoic acid thus split off, we next get rid of the acetic acid by repeated partial evaporations, taking care not to let the solution become too concentrated and thus perhaps injure the active principle. After the removal of the acetic acid the sulphuric acid is precipitated with the help of lead carbonate. The lead sulphate is removed and the filtrate is allowed to stand *in vacuo* over sulphuric acid until it has become of tarry consistency. It is then exhausted with ether, acetone and absolute alcohol in succession.

The residue is physiologically very active, and it gives all the reactions characteristic of an aqueous solution of the gland—a beautiful rose color on the addition of an alkali, a green color with ferric chloride, reducing silver nitrate, etc.

However, we are not yet dealing with a pure substance. If we neutralize an aqueous solution of the product thus obtained with a free alkali or with a carbonate of the alkalies, an overpowering odor much like that of coniine is perceived. As this odor passes that of pyridine seems to take its place. The basic substance thus liberated fumes in the air or when a glass rod moistened with hydrochloric acid is brought into its neighborhood. To remove this basic substance we made an aqueous solution of the active principle as above isolated, rendered it faintly alkaline with sodium carbonate and shook out with ether several times in succession. But this method does not remove all of the coniine-like substance. To effect this we were obliged to make the active principle strongly alkaline with sodium hydrate before shaking out with ether. The aqueous solution, in consequence of the addition of the free alkali, takes on a deep red color, and carmine red flocks are thrown out. After the removal of the coniine-like substance with ether, the fluid containing the red flocks was acidulated with sulphuric acid and the carmine-red substance was filtered off. The clear filtrate, which was a pale straw color, was concentrated *in vacuo*, and the active principle taken up in weak alcohol. On account of the insolubility of the sulphate of the active principle in absolute alcohol it is difficult to obtain this entirely free of sodium sulphate. We hope in future to remove both the coniine-like substance and the pigment with the help of ammonia. The presence of pyridine in our ammonia prevented our using it for this purpose.

This volatile base of a coniine-like odor has not hitherto been described as a constituent of the suprarenal capsule. The beautiful carmine-red pigment we take to be identical with the red substance which many other observers have found to be thrown out as a precipitate when ammonia is added to a concentrated aqueous solution of the gland, or when a solution is evaporated in the presence of lead acetate.* Others have believed this red precipitate to be the oxidation product of the active principle of the gland, but we shall presently see that the active principle is still present in the filtrate from the carmine-red body. We have as yet made no experiments with this pigment except to note that it is but slightly soluble in dilute alkalies and insoluble in dilute acids, and that iodine destroys the color. Our method appears to make the further study of this substance possible.

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* Holm, Journ. f. prakt. Chemie, c (1867), 150.

The coniine-like substance we have secured in the form of its hydrochlorate, and with it we have made certain studies which will be published at a later date.

CHARACTERISTICS OF THE BLOOD-PRESSURE-RAISING CONSTITUENT AS OBTAINED BY DECOMPOSING ITS BENZOYL COMPOUND.

We have now obtained the active principle in the form of a sulphate. As thus far isolated it is a hygroscopic, straw-colored residue which tends to crystallize on standing over sulphuric acid, agglomerates of small crystals forming on the edge of the bowl and the entire residue taking on a semi-crystalline appearance. This sulphate does not contain the volatile coniine-like substance, nor do we find the carmine-red pigment which falls out on the addition of an alkali. Alkalies no longer liberate the coniine-like substance, nor do they throw out the red pigment, but they cause a brownish discoloration, and on heating, alkaline vapors, probably ammonia, are given off.

Our sulphate is very active physiologically. A small quantity suffices to raise the blood-pressure as seen in Fig. 1, and it is therefore evident that we have isolated the active principle but slightly contaminated with other substances.

A final product even more active in small quantities is that whose action is shown in Fig. 2, where the mercury is driven out of the manometer. This more active compound still contains much of the coniine-like substance, and at once throws out the carmine-red pigment on the addition of alkalies. The sulphate of the coniine-like substance does not, as we have seen, raise the blood pressure; it therefore remains for us to consider whether the carmine-red substance which is precipitated on the addition of alkalies, either alone or in conjunction with small quantities of oxidizing agents, has any physiological significance or stands in any chemical relation to the blood-pressure-raising constituent. We incline to the opinion that this substance, which in the original aqueous solution of the gland gives with iodine water the "teinte rose-carmin tout à fait remarquable" of Vulpian, is in nowise connected with the blood-pressure-raising constituent. We have repeatedly removed or destroyed this substance by boiling solutions containing it with strong ammonia or with sodium hydrate, and have always been able to separate subsequently an active material. In proof of this we append Fig. 3, showing a great rise of blood pressure, although the material from which this active principle was obtained had been boiled for one hour with a 5 per cent. solution of sodium hydrate. After such treatment with alkalies, care must be taken in the subsequent evaporation of the alcohol with which the active principle is removed, to keep an acid reaction, as otherwise the greater part, if not all of it, will be destroyed by oxidation. And unless all evaporations are carried on under reduced pressure, much material will be lost, even with the above precaution.

Although somewhat contaminated with its own decomposition products, this final sulphate has all the characteristics of an active substance. As shown by repeated experiments, it promptly raises the blood pressure, it constricts the vessels of an inflamed eye, and when injected into the dorsal lymph sac of the frog it acts like a narcotic or cerebro-spinal poison.

As freed of the red substance the sulphate of the active principle behaves as follows: It is very soluble in water, fairly soluble in weak alcohol (50 per cent.), almost insoluble in absolute alcohol, and quite insoluble in ether, acetone, ligroine and chloroform. Its aqueous solution, even when freed from adherent sulphuric acid, has a slightly acid reaction. The addition of iodine water to a neutral solution does not give a rose-red color. Alkalies added to a strong solution give a brown color which deepens on heating. Ferric chloride gives a purplish brown, almost black in concentrated solution, which on the addition of tartaric acid and an alkali passes into a deep red color. Before the removal of the carmine-red substance the addition of ferric chloride gives the well-known emerald green color, which passes into red on the addition of an alkali.

It is evident that our sulphate gives Vulpian's ferric chloride reaction, though somewhat changed by the removal of what we take to be the chromogenic substance which gave his iodine reaction. It also promptly reduces silver nitrate in alkaline solution, but does not reduce Fehling's solution even on boiling.

PROBABLE RELATION TO THE ALKALOIDS.

More than a year ago, during our first studies with suprarenal extract, we were struck with the fact that every extract entirely free of proteids and physiologically active gave a fine pyrrol reaction when subjected to dry distillation. This is evidenced both by the odor and by the pine sliver reaction. A small quantity of the isolated sulphate also gives the pyrrol reaction when heated either alone or with zinc dust.

We attach considerable importance to this reaction. As is well known, alkaloids in general give pyrrol on dry distillation; morphine, for instance, on being heated with 10 parts of zinc dust gives off pyrrol, ammonia, trimethylamine, pyridine, phenanthrene, etc.* During the past winter we made several attempts to prove the presence of pyridine among the products of dry distillation of the active principle as above isolated, as its detection would prove that our principle was to be classed among the alkaloids.

In attempting to show the presence of pyridine we heated the active principle with zinc dust in a current of dry hydrogen and passed the distillation products through a test tube and two small flasks, the tube being placed in a cooling mixture, the first flask being partly filled with dilute sodium hydrate, and the second with dilute hydrochloric acid.

At the close of the experiment the test tube gave off alkaline vapors that smelled strongly of trimethylamine and had in addition a peculiar sweetish odor. The contents of the test tube diluted with water gave a precipitate with bromine water and with copper sulphate, but with the latter reagent the fine blue color characteristic of pyridine did not appear. The presence of amines seems to render difficult the detection of small quantities of pyridine. The contents of the third flask on exposure to the air took on a rose-red color and after a time threw out a precipitate of the same tint. Both flasks gave off the odor of benzaldehyde.

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* We are well aware that certain salts of glutaminic, pyromucic and its related acids also yield pyrrol on dry distillation. These compounds, however, like the proteids and their allies, appear to us to be excluded.

The following experiment was also made with a little of the sulphate, which no longer gave the rose-red reaction with iodine but which still contained a little of the coniine-like substance. For two hours it was kept at 150° C. in a sealed tube with 25 per cent. hydrochloric acid. On shaking out with ether a considerable crystalline residue was obtained which, without much purification, melted sharply at 120° C. and had all the properties of benzoic acid.

In the above decomposition experiments the quantity of material at command was small, perhaps not more than one-fifth of a gramme at a time, and not entirely pure. We do not, therefore, lay much stress on our failure to detect pyridine.

The fact that the nitrogen of our compound is given off in the form of amines and pyrrol gives strong ground to believe that our substance is to be classed with the pyridine bases, using this term in a broad way. Its basic character, its ability to take up acid radicles, as illustrated by the formation of a benzoyl product, its reducing power and its color reaction with ferric chloride are all points which can be urged in support of this view. Furthermore, cupric acetate, the serviceableness of which as a precipitant of carbo-pyridine bases has been shown by Gautier and Landi,* also precipitates our active principle; and iodine chloride, which Dittmar† found to give brown or yellow halogen addition-derivatives with a large number of the alkaloids, gives with a neutral or slightly acid solution of our active sulphate a brownish flocculent precipitate.

Of less weight in this connection, but nevertheless of some value, is the fact that its physiological action and its power in small doses is again in accord with what is known of alkaloids. Strychnine, thebaine and other alkaloids are readily called to mind as able to raise the blood pressure, and recently Tunnicliffe‡ has placed piperidine in the same list. The pyrrol* bases are also physiologically active, although less attention has been paid to them.

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* Compt. rend., cxiv (1892), 1154–1159.

† Berichte d. deutsch. chem. Gesellsch., xviii, 1612.

‡ Centralbl. f. Physiol., x, No. 25, 777.

PYROCATECHIN CANNOT BE SPLIT OFF FROM THE BLOOD-PRESSURE-RAISING CONSTITUENT.

As already said, Mühlmann has stated with great positiveness that the blood-pressure-raising constituent is pyrocatechin joined to some other substance, probably an acid. Mühlmann does not offer sufficient proof for his conclusion; and even had he proved the presence of pyrocatechin in the gland itself, this is a very different thing from showing that it enters into the chemical constitution of the blood-pressure-raising constituent.

We have repeated Mühlmann's work and have found that after boiling the medullary substance with hydrochloric acid, the ether takes up a trace of Vulpian's substance, as well as a substance that reduces Fehling's solution.

The ether residue also gives a precipitate with neutral lead acetate which, on decomposition with dilute sulphuric acid, sets free a compound that gives with ferric chloride the reaction so characteristic of pyrocatechin. Our blood-pressure-raising constituent is not precipitated by lead acetate and could not, therefore, have been responsible for this result.

Furthermore, on decomposing a fairly large quantity of the crude benzoyl product before referred to and shaking out the resulting benzoic acid with ether, we have twice found that the ether has taken up in addition to the benzoic acid a small quantity of a substance which reduces Fehling's solution and gives a precipitate with neutral lead acetate. On decomposing the resulting lead compound with dilute sulphuric acid and neutralizing the filtrate, we obtained a solution which behaved with ferric chloride in the way characteristic of pyrocatechin. The quantity of this lead compound obtained has been too small for analytical purposes.

We consider it, therefore, still uncertain whether or not a little pyrocatechin is present in the gland.

That the blood-pressure-raising constituent is, however, not a pyrocatechin derivative seems to us certain. To prove this we used, not the entire gland nor the medullary substance, but the active principle itself, as isolated by us by decomposing the benzoyl compound.

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*Jac. Ginzberg, Ueber das Verhalten des Pyrrols, etc., Inaug. Diss., Königsberg, 1890.

We have used large quantities of material representing in each experiment the active principle of several thousand glands. We have boiled this with hydrochloric acid of varying strength and for varying lengths of time, and in no case have we been able to find in the ether used as a solvent any trace of a substance that would reduce Fehling's solution or give a precipitate with lead acetate. Inasmuch as some of the chromogenic substance always passes into the ether from acid solutions, these two reactions are both necessary to establish the presence of pyrocatechin. In other words, we have failed to split off pyrocatechin from the active principle as isolated by us. If present, it could hardly have eluded our search, for even a minute quantity may be made to give a precipitate with neutral lead acetate.

We propose during the coming autumn to continue this work. Having made arrangements for the use of a large amount of material, we hope to secure both the benzoate and the sulphate in a form pure enough for analysis and for further chemical study.

SUMMARY.

We may summarize the results of our work as follows:

The blood-pressure-raising constituent of the suprarenal capsule may be completely precipitated from an aqueous extract by treatment with benzoyl chloride and sodium hydrate, according to the Schotten-Baumann method.

On decomposing the resulting benzoyl products, a residue is obtained which possesses great physiological activity. It gives the color reactions of Vulpian, reduces silver nitrate and possesses the other specific qualities of suprarenal extracts.

With the help of alkalies a carmine-red pigment may also be separated from these decomposition products. We take this pigment to be that one of the chromogenic substances of Vulpian which gives the rose-carmine color when suprarenal extracts are treated with oxidizing agents or alkalies.

A volatile, basic substance of a coniine-like odor is always found to accompany the crude benzoate. When these substances are removed the active principle is left as a highly active sulphate or hydrochlorate, as the case may be. It is therefore a basic substance. Its salts give a color reaction with ferric chloride; they also reduce silver nitrate, but not Fehling's solution.

It is not possible to split off pyrocatechin from this isolated active principle. The fact that dry distillation causes the appearance of amines and pyrrol in abundance, taken in connection with its ability to take up acid radicles, its reducing power, its precipitability by cupric acetate and iodine chloride, and its physiological action, lead us to conclude that our active principle is to be classed with the pyridine bases or alkaloids.

ADDENDUM, JUNE 15, 1897.

Since the foregoing paper was read before the Association of American Physicians on May 6, 1897, B. Moore has published a paper in the *Journal of Physiology*, vol. xxi, Nos. 4 and 5, May 12, 1897, entitled, "On the Chromogen and on the active Physiological Substance of the Suprarenal Gland." In this paper it is suggested that the active principle may be a pyridine derivative. Moore's conjecture is based, first, on a formula calculated by him from a combustion analysis of Krukenberg, made with an impure, hygroscopic, non-crystalline residue obtained by merely decomposing and drying a precipitate secured out of an alcoholic extract of the gland with the help of basic lead acetate. The calculated formula, the carbon content of which differs by 1.33 per cent. from that actually found by Krukenberg, is $C_5H_{14}NO_4$, and Moore points out that in it the ratio in which the elements carbon and nitrogen stand to each other is the same as in pyridine, C_5H_5N. Now such a calculation, though suggestive to the investigator, furnishes no proof of the presence of pyridine, for Krukenberg could not possibly have had in hand a chemical individual uncontaminated with other constituents of the gland. Krukenberg himself admits that his material was a mixture of unaltered pigment and its decomposition products. It is also certain that it was contaminated with other constituents of the gland. This is proved by the fact that basic lead acetate precipitates several of the constituents of the gland which pass into alcohol, and also by the fact that the analysed material contained 0.068 per cent. of sulphur and from 1.18 per cent. to 1.44 per cent. of ash.

A second reason given by Moore in support of his conjecture is that when "some suprarenal extract was cautiously fused with caustic potash so as to avoid charring, the peculiar odor of pyridine was at once obtained." He admits that proteids were present in this extract, and that pyridine and its homologues are formed in the destructive distillation of proteids, but states that the experiment was "conducted so as to avoid charring," and considers this a sufficient safeguard. This experiment has also no value, for the odor of pyridine can always be obtained when a mixture of dry, powdered proteid and powdered caustic potash are fused together so as to avoid charring. This experiment, therefore, must be made with material entirely free of proteids. Since reading Moore's paper we have fused a little of our active principle with caustic potash, but have failed to detect pyridine. If present in the molecule of the active principle, it is evident that larger quantities of the material must be used to prove it. Only indisputable chemical proofs can establish the presence of pyridine; the odor is not enough. We have frequently in various manipulations with the active substance met with what we took to be the odor of pyridine, but on shaking out with ether and adding an ethereal solution of hydrochloric acid and evaporating the ether we have been unable to detect pyridine in the residue.

DESCRIPTION OF THE TRACINGS.

Fig. 1. Tracing. Dog anæsthetized with morphine; curare and artificial respiration, both vagi cut. Injection of the active sulphate, freed from the carmine substance and coniine-like body, at ×.

A = running base line.

B = time record.

C = curve of the blood pressure, canula in the right carotid artery.

Fig. 2. Tracing. Conditions of the experiment as in Fig. 1. Injection at × of a highly active sulphate which still

gives the rose-carmine color with iodine water and still contains some of the coniine-like substance which does not raise the blood pressure. The blood pressure is so high that the mercury is driven out of the manometer.

Fig. 3. Tracing. Conditions as in 1. Injection at × of a material which is still highly active although it had been boiled with 5 per cent. sodium hydrate for one hour. In this experiment a number of injections had already been made; the pressure had fallen to the normal before the injection indicated at × was made.

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